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Bio-validation of bi-axial rotary thermal processing

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ABSTRACT

In continuous processing of canned particulate liquid foods, biological validation is necessary for lethality verification because of difficulties associated with gathering temperature history at the particles centers. In this study, carrot (d = 1.9 cm) and meat (d = 1.8 cm) alginate spherical particles, inoculated with spores of *Clostridium sporogenes* and *Geobacillus stearothermophilus*, respectively, were filled into cans along with a non-Newtonian liquid (carboxymethyl cellulose). Process simulations were used and time temperature profiles at the particles center were predicted for processing at three temperatures (110–125 °C) and two rotation speeds (5–25 rpm). Process times, B, were calculated to achieve an accumulated lethality of 3 and 15 min for carrot and meat alginate fabricated particles, respectively. These ranged from 19.5 to 36.4 min for carrot and from 19.3 to 40.1 min for meat alginate particles. Using spore counts of the particles before and after the given process, the number of log reductions, n, of the bacterial spores and hence the process lethality, F_o, was determined. Results indicated that there was no significant difference ($p > 0.05$) in F_o values between those obtained from biological validation and from numerical simulation, demonstrating that the standardized bio-validation technique can be effectively used for establishing/verification of thermal processing schedules.

1. Introduction

With today's consumers becoming more educated and health aware, the demand for convenient foods with high quality has boosted over time. Canning, in general, involves excessive thermal treatment of the food product, resulting in degradation of quality attributes (David, Graves, & Carlson, 1996, pp. 21–31). High temperature-short time (HTST) processing can be applied in order to improve the quality of the canned food products as quality factors are significantly more heat stable than the microorganisms. HTST processing includes aseptic processing, agitation processing and thin profile packaging. In agitation processing, mixing is enhanced inside the cans placed in rotary retorts, resulting in shorter processing time and better quality retention. The three common modes of rotation include end-over-end (EOE), fixed axial and free axial rotation. EOE involves rotating the secured car, with cans locked vertically within, around at different speeds giving the cans a vertical end over end rotation. In the fixed axial rotation, the rotation of the car is similar but the cans are placed within the car in a horizontal direction so that when the car rotates the cans get an axial agitation in a circular fashion. On the other hand, there are three phases of motion in a free axial rotation: fixed, transitional and free reel motion across the retort bottom. The fixed reel motion takes places over upper 220° of the cycle, the free rotation over the bottom 100° and the transitional phase on either side of the free rotation (20°). As the can rotates, the headspace bubble moves along the length of the can resulting in the agitation of the can content (Dwivedi & Ramaswamy, 2009).

In thermal processing, theoretical models can be useful tools for designing, optimizing and validating food systems; however, the usefulness of these models depends upon the accuracy of the input physical parameters, such as overall heat-transfer coefficient (U) and the fluid-to-particle heat-transfer coefficient (h_{fp}) (Sablani & Ramaswamy, 1996). In the liquid particulates heat transfer studies, it is necessary to have well-defined thermophysical properties for particles in order to obtain consistent heat transfer coefficients; that is why Nylon spheres were in general used instead of real food as there is variability in the thermophysical properties of the latter due to differences in the structure and chemical composition (Dwivedi & Ramaswamy, 2009).

In case of liquid particulate canned food products subjected to free axial rotation, temperature data collection is challenging due to difficulties involved in attaching the temperature measuring devices to the liquid and particles; therefore, biological validation is necessary to assure the safety of the thermal process (Chandarana

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& Unverferth, 1996). No work has been reported in the literature about using food alginate simulated particles in the biological validation of bi-axial rotation processing.

Contact method, which consists of immobilizing spores of heat resistant microorganisms in a matrix of alginate food puree forming a particle and then calculating process lethality, F_o, is the most commonly used technique in biological validation (Brown, Ayres, Gaze, & Newman, 1984). Inoculated food alginate fabricated particles can be utilized to determine the process lethality using initial and final spores' counts (Abdelrahim, 1994; Brown et al., 1984; Marcotte, Taherian, & Ramaswamy, 2000; Naim et al., 2008; Walsh, Isdell, & Noone, 1996). *Geobacillus stearothermophilus* and *Clostridium sporogenes* are heat-resistant and nonpathogenic marker organism that have been considered to follow similar thermal destruction profile to the target pathogen, *Clostridium botulinum* (Brown et al., 1984; Dallyn, Falloon, & Bean, 1977; Hassan & Ramaswamy, 2011c; Ocio, Fernandez, Rodrigo, & Martinez, 1996).

The objectives of the study were: (1) to match the heat behavior of carrot and meat alginate fabricated particles with an inert Nylon particle used extensively in our previous heat transfer studies (2) to use a numerical simulation model to predict time temperature profile at the alginate fabricated particle in order to calculate the process times using the time-temperature integration method (3) to fabricate spore entrapped carrot and meat alginate particles with known initial spore concentrations of *C. sporogenes* or *G. stearothermophilus* and (4) to determine the number of log reductions and hence the process lethality, F_o, using initial and final spore counts of fabricated particles (bio-validation) subjected to selected processing conditions, (5) to compare the F_o values from bio-validation with the model simulated values.

2. Materials and methods

2.1. Spore preparation

C. sporogenes and *G. stearothermophilus* spores preparation was done according to the methods used by Shao (2008) and Kim and Naylor (1966), respectively. Freeze-dried cultures of *C. sporogenes* (ATCC-7955) were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and stored at −40 °C. Spores preparation was done according to the method used by Shao (2008). The culture was hydrated in 10 ml Reinforced Clostridial Medium (RCM) broth (Oxoid, Basingstoke, Hampshire, UK) at 37 °C for 24 h under anaerobic conditions and stored at 4 °C. To prepare the inoculation culture, 0.1 ml of the broth was transferred to 50 ml of freshly prepared RCM and incubated at 37 °C for 24 h under anaerobic conditions. Two similar transfers were made in order to obtain a culture with viable count of approximately 10^6 CFU/ml. A volume of 0.2 ml of this culture was then transferred and spread on Campdem Sporulating Agar plate (CSA) and incubated at 37 °C for 7 days under anaerobic conditions in order to grow and form bacterial spores. The CSA medium contained 2.5 g tryptone (Oxoid, Basingstoke, Hampshire, UK), 2.5 g bacterial peptone (BD, Co., Spark, MD), 0.5 g Lab Lemco meat extract (Oxoid), 1 g yeast extract (BD, Co., Spark, MD), 0.028 g calcium chloride (BDH, Inc., Toronto, ON), 0.031 g $MnSO_4 \cdot H_2O$ (Fisher Scientific, Fair Lawn, NJ), 0.5 g glucose (Fisher Scientific) and 7.5 bacterial agar (Fisher Scientific) in 500 ml of distilled water. Spores were collected by flooding the agar plate surface while scrapping the colonies via sterile glass spreader. After harvest, the spores were washed three times by centrifuging at 4000× *g* for 15 min each at 4 °C and suspended in sterile distilled water to give approximately 10^6 CFU/ml. The spores' solution was stored at 4 °C until use (Shao, 2008).

Freeze-dried cultures of *G. stearothermophilus* (ATCC-10149) were obtained from the American Type Culture Collection (ATCC) and stored at −40 °C. Spores preparation was done according to the method used by Kim and Naylor (1966). The culture was hydrated in 10 ml TYG broth at 55 °C for 24 h under aerobic conditions in a shaking water bath (SW22, Julabo, Labortechnik GMBH, Germany). Two similar transfers were made in order to obtain a culture with viable count of approximately 10^8 CFU/ml. Then, the solution was stored at 4 °C. The TYG medium consisted of 5 g tryptone (BD, Co.), 2.5 g yeast extract (BD, Co.) and 1 g K_2HPO_4 (Acros Organics, NJ, USA) in 500 ml distilled water and the pH was adjusted to 7.2. Inoculation culture was prepared by transferring 1 ml of the cultured broth to 50 ml freshly prepared TYG broth and incubating at 55 °C for 24 h under aerobic conditions. A volume of 0.2 ml of this culture was then transferred and spread on a sporulation agar plate and incubated at 55 °C for 7 days under aerobic conditions in order to grow and form bacterial spores. The sporulation medium contained 4 g nutrient broth (BD, Co.), 2 g yeast extract (BD, Co.), 0.05 g $MnCl_2 \cdot 4H_2O$ (Acros Organics) and 10 g bacterial agar (Fisher Scientific) in 500 ml of distilled water. Spores were collected by flooding the agar plate surface while scrapping the colonies via sterile glass spreader. After harvest, the spores were washed three times by centrifuging at 4000× g and suspended in sterile distilled water to give approximately 10^8 CFU/ml. The spores' solution was stored at 4 °C until use.

2.2. Alginate particle making

Carrot alginate and meat alginate fabricated particles were fabricated according to the method used by Brown et al. (1984) and Marcotte et al. (2000). The sequence of fabrication steps is presented in Fig. 1. In biological validation studies, food alginate fabricated particles should be hard enough to save their integrity till the end of the thermal process. Also, they should have identical thermophysical properties to real food particles (Marcotte et al., 2000). Hassan and Ramaswamy (2011a, b) reported the optimum textural and thermophysical conditions for making carrot and meat alginate simulated particles. These conditions were sodium alginate concentration of 4.7 g/100 g, calcium chloride concentration of 2.6 g/100 g and immersion time of 32.0 and 30.8 h for carrot alginate and meat alginate fabricated particles, respectively.

2.3. Spores recovery and enumeration

Recovery of spores from the meat and carrot alginate particles was done according to the method used by Dallyn et al. (1977) and Brown et al. (1984). Particle was aseptically chopped up and the resulting small pieces were dissolved in 35 ml 5 g/100 g tri-sodium citrate in a 50 ml centrifuge tube with glass beads for approximately 20 min on a vortex mixer in order to assure a complete breakdown of the particles. Serial dilutions were made with 0.1 g/100 g peptone water and enumeration was done using the pour plate technique. Enumeration of *C. sporogenes* in carrot alginate fabricated particles was done in a modified PA3679 agar and the plates were incubated at 37 °C under anaerobic conditions for 5 days before counting (Ocio et al., 1996). Spores of *G. stearothermophilus* in meat alginate fabricated particles were enumerated in Tryptic soy agar (BD, Co.) plates, which were incubated at 55 °C under aerobic conditions for 5 days before counting (Shao, 2008). Initial count (N_o) in carrot alginate and meat alginate fabricated particles was found to be 10^5 and 10^7 CFU/ml, respectively.

2.4. Matching the heating rates of alginate particles with a Nylon particle

In previous heat transfer studies conducted to investigate the effect of process and product parameters on the rate of heat